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GENE THERAPY: Twenty-First Century Medicine

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■ **Abstract** Broadly defined, the concept of gene therapy involves the transfer of genetic material into a cell, tissue, or whole organ, with the goal of curing a disease or at least improving the clinical status of a patient. A key factor in the success of gene therapy is the development of delivery systems that are capable of efficient gene transfer in a variety of tissues, without causing any associated pathogenic effects. Vectors based upon many different viral systems, including retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses, currently offer the best choice for efficient gene delivery. Their performance and pathogenicity has been evaluated in animal models, and encouraging results form the basis for clinical trials to treat genetic disorders and acquired diseases. Despite some initial success in these trials, vector development remains a seminal concern for improved gene therapy technologies.

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INTRODUCTION

Gene therapy is a form of molecular medicine that has the potential to influence significantly human health in this century. It promises to provide new treatments for a large number of inherited and acquired diseases (1). The basic concept of gene therapy is simple: introduce into target cells a piece of genetic material that will result in either a cure for the disease or a slowdown in the progression of the disease. To achieve this goal, gene therapy requires technologies capable of gene transfer into a wide variety of cells, tissues, and organs. One of the biggest stumbling blocks to successful widespread application of such genetic treatments is the development of safe and effective vectors with which to ferry genetic material into a cell.

The process of gene delivery and expression is known as transduction. Successful transduction requires overcoming a number of obstacles that are common to all vector systems (2). The first issue to be addressed is that of production. An ideal vector should be one that can be produced in a highly concentrated form, using a convenient and reproducible production scheme. This has been a challenge for many of the currently used vector systems, but in many cases creative approaches have overcome this barrier. The vector must be capable of targeting the cell type most appropriate for the disease, whether it be dividing or nondividing cells. Understanding of the transduction process through studies of vector uptake, intracellular trafficking, and gene regulation has facilitated the development of efficient vehicles for gene delivery. In many cases it would be desirable to achieve stable, sustained gene expression, which requires either integration of the vector DNA into the host DNA or maintenance as an episome. When using integrating vector systems, it is important to consider the potential hazards of insertional mutagenesis, and thus vectors capable of site-specific integration will be attractive. In many cases, expression of the therapeutic gene will require exquisite regulation, and thus the transcriptional unit must be capable of responding to manipulations of its regulatory elements. Finally, no pathogenic or adverse effects should be elicited by vector transduction, including undesirable immune responses.

Vectors that have been developed to overcome these obstacles fall into two broad categories: nonviral and viral vectors (1). The nonviral vectors consist of naked DNA delivered by injection, liposomes (cationic lipids mixed with nucleic acids), nanoparticles, and other means. Although nonviral vectors can be produced in relatively large amounts and are likely to present minimal toxic or immunological problems, presently they suffer from inefficient gene transfer. In addition, expression of the foreign gene tends to be transient, precluding their application to many diseased states in which sustained and high-level expression of the transgene is required. It is likely that future gene therapy protocols will use novel innovations to improve on the efficiency of nonviral vector systems, often building upon observations from viral vector transduction. Viral vectors are derived from viruses with either RNA or DNA genomes and are represented as both integrating and nonintegrating vectors. The former holds the promise of lifelong expression of the deficient gene product. Efficient gene transduction can also be achieved from vectors that are maintained as episomes, especially in nondividing cells.

VIRAL VECTORS

The basic concept of viral vectors is to harness the innate ability of viruses to deliver genetic material into the infected cell. In general, the major preoccupation of viruses is to replicate and produce copious amounts of progeny. Most viruses gain little by killing the host, but unfortunately many viral infections lead to deleterious effects on the host, accompanied by destruction of infected host cells. Damaging effects can be caused by induction of genes whose products are hazardous to the host or by acquiring host genomic material that can lead to pathogenesis. The basic principle of turning these pathogens into delivery systems relies on the ability to separate the components needed for replication from those capable of causing disease (see Figure 1). The first step of viral vector design is, therefore,

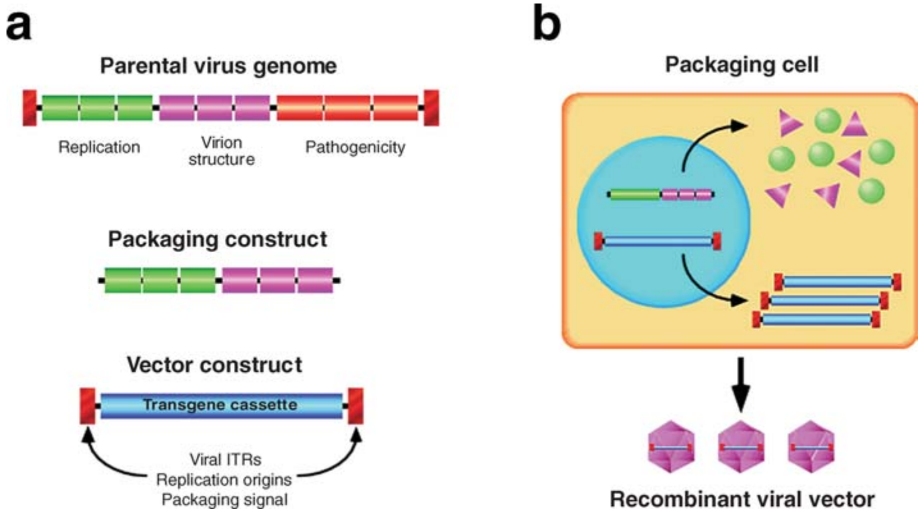
a
Parental virus genome
Replication
Virion structure
Pathogenicity
Packaging construct
Vector construct
Transgene cassette
Viral ITRs
Replication origins
Packaging signal
b
Packaging cell
Recombinant viral vector

Figure 1 Principle of generating a viral vector. (*a*) Converting a virus into a recombinant vector. Schematic of a generic viral genome is shown with genes that are involved in replication, production of the virion, and pathogenicity of the virus. The genome is flanked by *cis*-acting sequences that provide the viral origin of replication and the signal for encapsidation. The packaging construct contains only genes that encode functions required for replication and structural proteins. The vector construct contains the essential *cis*-acting sequences and the transgene cassette that contains the required transcriptional regulatory elements. (*b*) The packaging and vector constructs are introduced into the packaging cell by transfection, by infection with helper virus, or by generating stable cell lines. Proteins required for replication and assembly of the virion are expressed from the packaging construct, and the replicated vector genomes are encapsidated into virus particles to generate the recombinant viral vector.

to identify the viral sequences required for replication, assembly of viral particles, packaging of the viral genome, and delivery of the transgene into the target cells. Next, dispensable genes are deleted from the viral genome to reduce replication and pathogenicity, as well as expression of immunogenic viral antigens. The gene of interest together with transcriptional regulatory elements (referred to as the transgene) are inserted into the vector construct, and a recombinant virus is generated by supplying the missing gene products required for replication and virion production. The more genes that are removed from the virus, the more replication defective the vector will be, and there is less chance of recombination to generate the infectious parental virus. The nature of the virus biology will usually determine the means of production. For example, retroviruses are produced in packaging cell lines, and vector particles accumulate in the culture medium. In contrast, adenovirus and adeno-associated virus (AAV) vectors are generally produced from transfections, and cells must be lysed to liberate the viral particles. In this review, we describe the salient features and applications of some of the most commonly used viral vectors. There are a number of other emerging vector systems that are still in their infancy and have been extensively discussed in other excellent reviews (1, 3).

RNA VIRUS VECTORS

The most commonly used RNA virus vectors are derived from retroviruses, and these were among the first viral delivery systems to be developed for gene therapy applications. Retroviruses are a large family of enveloped RNA viruses found in all vertebrates, and they can be classified into oncoretroviruses, lentiviruses, and spumaviruses.

Retroviruses

The enveloped virus particle contains two copies of the viral RNA genome, surrounded by a cone-shaped core (for an in-depth review of retrovirus biology, see Reference 4). The viral RNA contains three essential genes, *gag*, *pol*, and *env,* and is flanked by long terminal repeats (LTR). The *gag* gene encodes for the core proteins capsid, matrix, and nucleocapsid, which are generated by proteolytic cleavage of the *gag* precursor protein. The *pol* gene encodes for the viral enzymes protease, reverse transcriptase, and integrase, which are usually derived from the *gag-pol* precursor. The *env* gene encodes for the envelope glycoproteins, which mediate virus entry. Oncoretroviruses are simple viruses encoding only the structural genes *gag*, *pol*, and *env*, whereas lentiviruses and spumaviruses have a more complex organization and encode for additional viral proteins (see Figure 2).

After binding to its receptor, the viral capsid containing the RNA genome enters the cell through membrane fusion. The viral RNA genome is subsequently converted into a double-stranded proviral DNA by the viral enzyme reverse

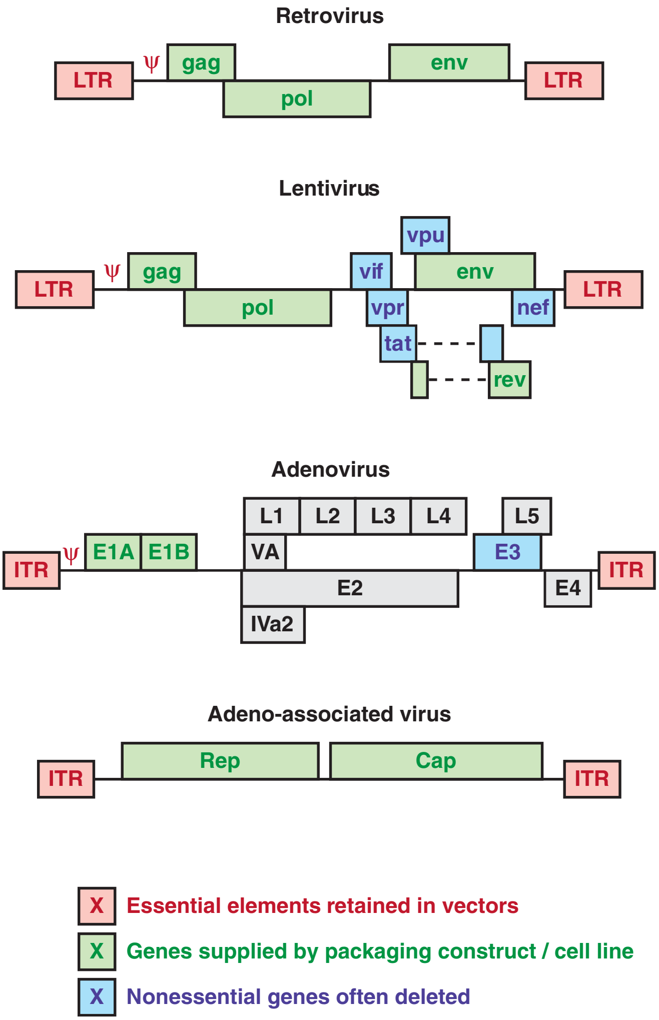
Retrovirus
LTR
ψ
gag
pol
env
LTR
Lentivirus
LTR
ψ
gag
pol
vif
vpu
vpr
tat
env
nef
rev
LTR
Adenovirus
ITR
ψ
E1A
E1B
L1
L2
L3
L4
L5
VA
E2
IVa2
E3
E4
ITR
Adeno-associated virus
ITR
Rep
Cap
ITR
X Essential elements retained in vectors
X Genes supplied by packaging construct / cell line
X Nonessential genes often deleted

Figure 2 Schematics of the commonly used viruses that are converted to recombinant viral vectors. The colored boxes indicate genes or *cis*-acting elements that are either essential [and therefore retained in vectors (*red*) or supplied by packaging constructs or cell lines (*green*)] or that are nonessential and often deleted (*blue*). Only the major genetic elements are shown, and viruses are not drawn to scale. In second- and third-generation derivatives of each vector system, more viral genes are deleted (see text for details).

transcriptase. The proviral DNA is associated with viral proteins, including nucleocapsid, reverse transcriptase, and integrase, in a preintegration complex and translocates to the nucleus, where the integrase mediates integration of the provirus into the host cell genome. Host cell transcription factors initiate transcription from the LTR, and new viral particles are formed at the plasma membrane. *Gag-pol* and *gag* precursors assemble together with two copies of viral RNA, and *env* glycoproteins are incorporated into the viral membrane during the budding process. In the newly formed virion, *gag* and *gag-pol* precursors are subjected to processing by the viral protease, which results in maturation of the virion.

ONCORETROVIRAL VECTORS These vectors have been derived from a number of different oncoretroviruses, including murine leukemia virus (MLV), spleen necrosis virus, Rous sarcoma virus, and avian leukosis virus. Replication-defective MLV vectors are generated by replacing all viral protein encoding sequences with the exogenous promoter-driven transgene of interest. In addition to the packaging signal, the vectors retain viral LTRs and adjacent sequences, which are essential for reverse transcription and integration. Vector RNA production is either driven by the U3 region of the LTR or by a CMV/LTR hybrid with higher transcriptional activity. In these vectors, the 3′ U3 region of the LTR is intact and is copied over to the 5′ LTR during reverse transcription, allowing efficient integration and LTR-driven transgene expression in the transduced cell.

Packaging of retroviral vectors is achieved by providing the structural proteins in *trans* in the packaging cells. The first packaging cell lines expressed *gag*, *pol*, and *env* from a complete proviral DNA, lacking only the packaging signal (5). However, sequence homology between the vector and packaging constructs facilitated recombination, allowing generation of a replication competent virus. To prevent recombination, packaging cells have been developed that express *gag/pol* and *env* from separate constructs. Furthermore, expression from the packaging constructs is no longer driven by the viral LTR but by constitutive promoters that allow a high level of virus production (5, 6). To avoid the laborious and time-consuming practice of generating cell lines, high-titer vectors can be produced by transient transfection (7). Viruses are recovered from the supernatants of actively growing producer cells.

Major concerns in the use of retroviral vectors are the possibility of vector mobilization and recombination with defective (endogenous) retroviruses in the target cell. This prompted the development of self-inactivating vectors (8). In these vectors LTR-driven transcription is prevented in transduced cells by deletion in the U3, and transgene expression is driven instead by an internal promoter, allowing the use of regulated and tissue specific promoters.

LENTIVIRAL VECTORS In addition to *gag*, *pol*, and *env*, lentiviruses encode three to six additional viral proteins, which contribute to virus replication and persistence of infection (for in-depth reviews of lentiviruses, see References 9 and 10). Two of the accessory proteins, *tat* and *rev*, are present in all lentiviruses and mediate transactivation of viral transcription and nuclear export of unspliced viral RNA, respectively. Although vectors based on Simian, Equine and Feline lentiviruses have been developed (11), we focus on human immunodeficiency virus type 1 (HIV-1)-based vectors because they have been most extensively studied.

In addition to *gag*, *pol*, and *env*, HIV-1 encodes six accessory proteins (*tat*, *rev*, *vif*, *vpr*, *nef*, and *vpu*). The retroviral vector design provided an excellent template for development of lentiviral vectors. The HIV-1-based lentiviral vectors are devoid of all viral sequences apart from essential *cis*-acting sequences, including the LTRs and the packaging signal (see Figure 2). The *rev* responsive element (RRE) is also included in the vector RNA. The viral *rev* protein is provided in *trans* to ensure efficient nuclear export of the full-length viral RNA genomes through binding to the RRE. Initially the endogenous LTR was used to drive vector RNA expression via transactivation by the *tat* protein (12), but further generations of the vector utilize a CMV/LTR hybrid promoter, which increases vector production and allows vector production to be independent of *tat* expression (13). The accessory genes, *vif*, *vpr*, *nef*, and *vpu*, are dispensable for lentiviral vector production and transduction, and they are deleted from the packaging construct (13). The central poly purine tract (cPPT), from the *pol* ORF, can be included in *cis* to improve nuclear import of the proviral DNA and hence accelerate transduction (14, 15). Furthermore, the biosafety of vectors is improved by the development of self-inactivating vectors, which are less likely to be mobilized following infection with HIV (16, 17).

The HIV-1 *env* glycoprotein has a highly restricted host range in that it infects cells containing CD4 and coreceptors. To broaden the host range of lentiviral vectors, they can be pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) *env*, which is provided in *trans* and imparts a wide tropism (12). Vectors are harvested from the supernatant, and those pseudotyped with VSV-G can be concentrated to produce high-titer preps. Titers can be determined using assays that measure the amount or activity of proteins incorporated in the vector particles, such as the p24gag ELISA assay. Stable packaging cell lines have now been developed, in which the producer cells express the structural proteins from minimal packaging constructs and expression is driven by an inducible promoter to minimize the toxicity of the VSV-G envelope protein (18, 19). Other viral glycoproteins have also been used to pseudotype lentiviral vectors and provide altered cell tropism (20, 21).

FOAMY VIRAL VECTORS Foamy viral (FV) vectors have recently been developed and are quite similar to retroviral and lentiviral vectors (22). The FV genome contains three additional ORFs (*tas/bel1*, *bel-2*, and *bel-3*), with *tas/bel1* being the coactivator of viral transcription (23). In addition to the packaging signal that consists of the 5′-untranslated region and the 5′ portion of the *gag* ORF present in all retroviral vectors, FV vectors contain the 3′ region of the *pol* ORF, which is critical for efficient packaging of these vectors. Similar to other retroviral vectors, the 5′ U3 region of the LTR in the vectors' plasmid can been replaced by a CMV promoter, which increases vector expression and makes vector production independent of *tas/bel1*.

FV vectors are produced by transient transfection of the vector construct as well as the packaging constructs encoding for the structural proteins *gag*, *pol*, and *env* in 293T cells (22). Because the FV envelope has a broad cellular host range, it is used by the vector, and therefore, the *env* sequences are included in the packaging construct or expressed from a separate construct. The FV *env* contains, in contrast to other retroviruses, an ER sorting signal, which allows FV particles to bud from intracellular membranes, and therefore, the majority of the infectious virions is cell associated (24). Consequently, the infectious particles have to be released from the packaging cells by a freeze-thawing process.

Because retroviral vectors were some of the first to be utilized for gene delivery, they have been extensively used in many applications (2, 25). Their ability to integrate and provide long-term gene expression has made them particularly useful for generating stable cell lines that express a transgene of choice and for marking studies of cell lineage. Their dependence upon cell division has restricted in vivo applications to gene delivery in actively dividing tissues, such as stem cells and cancer cells. In contrast, lentivirus vectors have been used for gene delivery in vivo by direct administration in many organs, including brain, eye, liver, and muscle (26). They have also been used for ex vivo transduction of hematopoietic cells, followed by bone marrow transplantation (27).

The limited cellular tropism of the natural envelope of wild-type viruses is one of the barriers for retroviral transduction. However, retroviruses have the ability to incorporate *env* glycoproteins from related as well as unrelated viruses, thus allowing pseudotyping with alternative glycoproteins. A number of different envelopes have been used to generate pseudotyped retroviral vectors with broad host ranges, including the VSV-G glycoprotein or the amphotropic MLV envelope. Pseudotyping also allows transfer of specific tropisms to the vector. Neurotropism and retrograde axonal transport was accomplished by the vector via pseudotyping with the G protein of Mokola lyssaviruses (28), and the filovirus (Ebola Zaire) envelope supported transduction of airway epithelia (21). Interestingly, the entry pathway of the retroviral vector has minimal effect on the transduction efficiency.

Reverse transcription and nuclear translocation of the preintegration complex are thought to be limiting steps in retroviral transduction, especially in terminally differentiated postmitotic cells. Proviral DNA synthesis of all retroviruses depends strongly on cellular conditions, and low nucleoside pools or absence of cellular cofactors might explain the incomplete reverse transcription in quiescent or stationary cells. In contrast to other retroviral vectors, FV vector particles can contain fully reverse-transcribed viral DNA, owing to activation of the process of reverse transcription before virus assembly. This suggests that FV vector gene transfer might be more efficient in certain postmitotic cells in which reverse transcription is limited.

Because HIV-1 is a human pathogen, there are biosafety concerns about the use of HIV-1-based lentiviral vectors. The current HIV-1 lentiviral vector system is depleted of all accessory proteins, and viral sequences in the vectors have been minimized. Thus, the replication of these vectors is highly disabled, and the possibility of homologous recombination is minimized. In addition, codon optimization of the packaging construct further decreases the risk of homologous recombination. The use of vectors based on other lentiviruses might eliminate some of the concerns (11); however, the risk associated with the introduction of nonhuman lentiviral vectors in human tissues is unknown.

DNA VIRUS VECTORS

Of the vectors derived from viruses with DNA genomes, the most prominent are those based on adenovirus (Ad) and the adeno-associated virus (AAV). Adenoviruses contain a double-stranded DNA (dsDNA) genome of ~36 kb, whereas AAVs consist of a single-stranded DNA molecule that is relatively small (~4.7 kb). The basic principals of vector design (Figure 1) also apply to vectors derived from DNA viruses. However, there are important practical differences in terms of construction, production, and purification of these vectors.

Adenovirus

Ads have been isolated from a large number of species and tissue types (for an in-depth review of Ad biology see Reference 29). The human Ad family consists of more than 50 serotypes that can infect and replicate in a wide range of organs, such as the respiratory tract, the eye, urinary bladder, gastrointestinal tract, and liver. The Ad genome consists of a double-stranded linear DNA molecule (size: ~36 kb) with overlapping transcription units on both strands. Extensive splicing results in the production of over 50 proteins; 11 of which are structural virion proteins. The viral life cycle occurs in an early and a late phase, divided by the onset of viral DNA replication. Adenoviral genes fall into three major groups, depending on the time course of their expression during the viral replicative cycle: early (E1A, E1B, E2, E3, and E4), delayed (IX and IVa2), and the major late transcription unit (see Figure 2). The latter is processed into five mRNAs (L1–L5) that share the same carboxy terminus. These transcription units are transcribed by the cellular RNA polymerase II, whereas the viral-associated (VA) RNA is transcribed by RNA polymerase III. The viral genome contains two identical origins for DNA replication within each terminal repeat. The E2 region encodes proteins required for replication, including the viral polymerase, and proteins from the E1 and E4 regions also contribute to efficient DNA replication. The gene products of the E3 region are involved in immune surveillance and suppression but are nonessential for infection in vitro.

The Ad genome is packaged in a nonenveloped icosahedral protein capsid. The fiber protein projects from the virion, and the carboxy-terminal knob domain forms a high-affinity complex with a host cell surface receptor protein. For the majority of Ad serotypes this receptor is the Coxsackie-adenovirus receptor (CAR) (30). In addition to attachment, efficient virus internalization requires an interaction between the viral penton base and the cellular integrin α_v receptor (31). After entry, the virus rapidly escapes from the endosome, and transport to the nucleus is accompanied by gradual disruption of the virus particle (32). The viral genome is imported through the nuclear pore and associates with the nuclear matrix to facilitate initiation of the primary transcription events (33). The Ad genome is transcribed and replicated at discrete replication centers in the nucleus of the infected cell (34), and the viral DNA does not normally integrate into the host genome.

Adenoviral infection causes an initial nonspecific host response with synthesis of cytokines (tumor necrosis factor as well as interleukin 1 and 6), followed by a specific response of cytotoxic T lymphocytes directed against virus-infected cells that display viral peptide antigens (29). In addition, there is activation of B cells and the necessary CD4-positive T cells, leading to a humoral response. Serologic surveys found antibodies against Ad serotypes 1, 2, and 5 in 40% to 60% of children. The immune response of the host against adenoviral proteins is the major hurdle to the efficient and safe use of adenoviral vectors.

Most adenoviral vectors are derived from Ad serotype 5; however, Ad vectors have also been generated from other serotypes, including human Ad2, Ad7, and Ad4 as well as nonhuman viruses. Replication-defective Ad vectors are designed by replacing crucial adenoviral coding regions (35). In the first generation of Ad vectors, the E1 gene was replaced with the transgene (36). Because E1A is the principal protein that activates the expression of other Ad transcription units' genes (37), and other E1 proteins play crucial roles in viral replication, these vectors are replication defective on most cell lines. E1-deleted vectors can be propagated in cell lines that provide the E1 gene products in *trans*, such as the human 293 cell line (38). Transgenes of up to 4.7–4.9 kb can be incorporated into E1-deleted adenoviral vectors (39). The cloning capacity of Ad vectors can be further increased by deletion of additional dispensable sequences from the Ad genome, such as the nonessential E3 region (40). Combining the E1 and E3 deletions provides a total cloning capacity of 8.3 kb in one mutant virus. Newly developed E1-complementing cell lines with a minimal amount of viral sequences help reduce the chances of homologous recombination between the vector and the host genome, and thus, less replication-competent Ad is generated (41, 42). Vectors can be produced at titers of up to 10^{13} particles/ml and are purified by cesium chloride gradient ultracentrifugation or column chromatography.

Although E1-deleted viruses are defective for replication, in some cell types they can produce virus proteins that serve as foreign antigens to induce a cellular immune response. Many attempts have been made to reduce immunogenicity by engineering the second generation of Ad vectors that are additionally deleted in other viral transcription units, such as E2 and E4. Because the E2 region encodes proteins that are essential for replication of the viral chromosome, it has to be provided in *trans* in the packaging cells. A number of deletions have been introduced into the E4 region, which encodes proteins required for efficient viral DNA replication and late protein synthesis. Partial deletions of the E4 region can produce viable vectors, and E4-complementing cell lines have been developed for more extensive deletions (43–46). Although deletions in the E4 region increase the cloning capacity of the Ad vector, some reports indicate that the E4 region may exert a positive effect on long-term expression (47–49).

Theoretically it should be possible to reduce viral genes to a minimum and create "gutted" vectors that carry no viral sequences, apart from the inverted terminal repeats (ITRs) and the *cis*-acting packaging signal (50). These vectors require helper viruses for propagation, generating a problem in the purification of a helper-free virus. An important step toward a third generation of Ad vectors was the development of high-capacity, helper-dependent vectors based on the Cre/*loxP*-system of site-specific DNA excision (51–53). Using the Cre*loxP*-system, 25 kb of adenoviral genome can be deleted from an Ad vector containing *loxP* sites in 293 cells stably expressing the Cre recombinase (52). Alternatively, the packaging signal can be deleted from the Ad helper virus (51, 54). These vectors have a great potential for efficient gene delivery and long-term gene expression, although production is very laborious and the incoming virus particles can still stimulate a deleterious immune response (50, 55).

Adeno-Associated Virus

AAV is a nonpathogenic human parvovirus (for an in-depth review of parvovirus biology see Reference 56). Productive AAV infection requires helper functions that can be supplied by coinfection with helper viruses, such as Ad and herpesvirus. AAV can also replicate in cells that have been put under stress, such as irradiation or treatment with genotoxic agents. In the absence of a permissive environment that will support AAV replication, the viral DNA can become integrated into the host chromosomal genome to establish a latent infection (reviewed in Reference 57).

Many different serotypes of AAV have been isolated, and the list continues to increase (58–63). They have in common a similar size and genomic configurations of replication and structural genes. AAV serotype 2 (AAV2) is the best characterized and has been the most frequently employed recombinant AAV (rAAV) vector. AAV virions are small nonenveloped particles (20–25 nm) that carry a linear single-stranded DNA (ssDNA) genome, which is approximately 4.7 kb in size. There are 2 viral ORFs, *rep* and *cap*, flanked by T-shaped ITRs. The ITRs are important for replication, packaging, and integration, and these are the only genetic elements from the virus that are retained in rAAV vectors (see Figure 2). There are four regulatory Rep proteins that are required for replication and packaging. The *cap* ORF encodes for three structural proteins (VP1, VP2, and VP3) that form the capsid.

The different serotypes of AAV utilize a variety of approaches for cell entry, and this results in different host ranges. The primary attachment site for AAV2 is the ubiquitous heparan sulfate proteoglycan (64). The fibroblast growth factor receptor 1 and integrin $\alpha_v\beta_5$ have both been implicated as coreceptors that facilitate internalization by endocytosis (65, 66). AAV4 and AAV5 use sialic acid, although different carbohydrate linkages determine specificity (67) and the PDGF receptors are also involved in AAV5 infection (68). After binding to its receptor, the virus enters the cell through receptor-mediated endocytosis and is subsequently transported to the nucleus (69, 70). Viral uncoating in the nucleus releases the single-stranded genome that then needs to be converted to a double-stranded form to enable gene expression.

The self-complementary sequences in the ITRs fold back on themselves to form hairpin structures that contain the replication origins. The large viral Rep proteins bind to a specific sequence within the ITRs and mediate replication (56). In addition, the virus relies upon the host cell replication machinery as well as helper functions supplied by the coinfecting virus. The helper proteins promote initiation of transcription, viral gene expression, and DNA replication. Replication proceeds at discrete sites in the nucleus (71), and strand displacement produces both plus and minus genomes that are packaged with equal efficiency into the icosahedral capsid.

The AAV viral vector system was initially developed by replacing the viral *rep* and *cap* genes with transgene sequences. The vector genome can be rescued by supplying Rep and Cap proteins in *trans* from a packaging plasmid, together with a helper virus infection (72, 73). Production of wild-type virus by homologous recombination has been minimized by reducing overlap between the vector and packaging plasmids. Although high-titer AAV vectors can be produced using this system, wild-type Ad contaminates these vector preparations. Knowledge of the helper requirements from the Ad genome enabled the development of plasmid-based systems in which transfection of mini-Ad plasmids into 293 cells (expressing Ad E1 proteins) supplies all the helper proteins (E2A, E4, and the VA RNA) without production of infectious Ad (74, 75). Alternative approaches include generating packaging cell lines or recombinant viruses (either Ad or HSV-1), containing elements from AAV required for replication and packaging (76–78). Baculoviruses have also been developed for production of vectors on large scales in insect cells (79). Knowledge of the receptors has aided the development of column chromatography approaches to purification for different serotype vectors.

AAV vectors based on the serotype 2 capsid have been the most commonly used for gene therapy studies and have demonstrated transduction in a large number of cell types and experimental model systems (reviewed in References 80, 81). The vector can transduce nondividing cell types and has been used in muscle, retina, brain, liver, and lungs. There is initially a slow rise in gene expression levels over the first few weeks after in vivo administration, and then a stable plateau is reached (82). The exact reason for this delay in gene expression is not exactly clear. It may reflect requirements for cytoplasmic trafficking, vector uncoating, and conversion of the incoming ssDNA genome into a dsDNA form capable of gene expression. This step is mediated by the host cell machinery and probably occurs by second-strand synthesis. AAV transduction can occur independently of the cell cycle; however, transduction efficiency is markedly improved in cells during S phase (83). Furthermore, activation of the cellular DNA repair machinery also supports second-strand synthesis, thus improving AAV transduction (84, 85). Some of the alternative serotype capsids give quicker transduction than AAV2 vectors (86). Transduction can also be obtained with faster kinetics using vectors with genomes half the size of wild type, which are thought to reanneal through self-complementation, independently of DNA synthesis (87).

One of the major limitations for the use of AAV as a gene delivery vehicle is the relatively small packaging capacity. The unique ability of AAV vectors to become joined into concatamers by head-to-tail recombination of the ITRs has been exploited as a means to increase the coding capacity (88). In this approach, either the gene itself or the different elements of the transgene expression cassette are split over two AAV vectors that are administered simultaneously (89, 90). Transgene expression is obtained only after recombination between the two viral genomes, but the efficiency is often reduced as compared to single vector transduction.

The AAV vectors do not contain any viral coding regions, and therefore, there is no toxicity associated with gene expression, However, a single injection of AAV vector elicits a strong humoral immune response against the viral capsid, which will interfere with readministration of the vector (91, 92). Furthermore, natural infections have resulted in a high prevalence of circulating neutralizing antibodies against AAV in the majority of the population, which may inhibit transduction. The use of AAV vectors containing cap proteins from different serotypes may overcome the problems of neutralizing antibodies (91), as may modifications to the virus capsid (93).

Herpesvirus

Human herpesviruses are a class of large DNA viruses with double-stranded genomes capable of accommodating a large amount of foreign DNA (for an in-depth review of herpesvirus biology see Reference 94). Herpes simplex virus type 1 (HSV-1) has been developed as a vector for gene delivery (95). The HSV-1 virion is about 20 nm in diameter and consists of four components: envelope, tegument, capsid, and viral genome. The envelope is derived from the cellular membrane and contains approximately 12 viral glycoproteins essential for viral entry. The tegument is the protein layer between the capsid and the envelope, and this layer contains at least 10 viral proteins, which are involved in the shutoff of host protein synthesis as well as in the activation of immediate early viral gene expression and assembly functions. Among these are proteins called VP16 (essential for trans-activation and virion envelopment), VP22 (membrane translocation domain), and virion host shut-off (vhs) protein. The icosahedral capsid consists of seven viral proteins and contains the linear dsDNA genome, which is 152 kb in size and is divided into unique long (U_L) and unique short (U_S) regions that are flanked by terminal repeats. The virus encodes at least 80 viral proteins with very little splicing of genes. A natural infection can result in either lytic replication in mucosal or epithelial cells or in a latent state in neurons with persistence of the virus genome (94).

Four of the HSV-1 glycoproteins in the virus envelope mediate interaction with the host cell and virus entry. The gB and gC proteins bind to heparan sulfate on the cell surface, and this is followed by gD binding to cellular receptors known as herpesvirus entry mediators HVEM/HveA and HveC/nectin-1. The gB, gD, and gH/gL complexes are all thought to be required for fusion of the virus envelope with the cell membrane to allow release of the nucleocapsid into the cellular cytoplasm. During the lytic replication cycle, the host cell protein synthesis is shutoff immediately after infection by the tegument vhs protein. Subsequently, the viral capsid releases the viral DNA into the nucleus, where it will circularize. Within several hours after infection, protein expression from the circular genome is initiated. Protein expression occurs in a highly regulated fashion and can be divided into three groups of sequentially expressed proteins: α- or immediate early genes, β- or early genes, and γ- or late genes. Once β-gene products are present, DNA replication and γ-gene production are initiated, and progeny virus will be produced. In the nucleus, capsid proteins assemble together with the viral DNA genomes, and the newly formed viral capsids bud through the nuclear membrane. On their way to the Golgi apparatus, the virion obtains the tegument and the viral envelope, and subsequently the virus is released from the cell through secretory vesicles.

Virus replication in the permissive epithelial cells produces virions that can enter the nervous system through axon terminals of local sensory neurons to enable establishment of a latent infection. Upon entry, the virus nucleocapsid is transported to the nucleus in the cell body by retrograde transport along the axon. The viral genes, which are involved in the establishment of a latent infection, are currently unknown, but de novo viral protein synthesis is not required. However, latency is related to the expression of latency-associated transcripts (LAT), which are expressed from a promoter that is highly active in neurons (96). The virus persists in the latent state for the lifetime of the infected individual. Reactivation of the latent virus can be induced by different stimuli, such as stress and UV irradiation, and results in production of progeny virions that are transported along axons to establish an active infection at the primary site of infection.

Replication-deficient HSV-1 vectors have been developed that do not express lytic genes and can result in quiescent and persistent genomes in neurons (97). It is relatively easy to manipulate and engineer the virus genome by recombination to insert transgenes and delete virus genes involved in lytic growth or toxicity. Two different viral vector systems have been developed to generate either recombinant HSV-1 vectors or HSV-derived amplicon vectors. Recombinant HSV-1 vectors contain a number of deletions in the α-genes and the vhs, and they can harbor large transgenes up to 30 kb in size (98). These vectors are nonreplicating and can be propagated to high titers in complementing cell lines that provide the essential α-genes in *trans*. Because many of these virus genes are toxic, they are often expressed in an inducible fashion in complementing cells. Recombinant HSV-1 vectors still retain large proportions of the HSV-1 genome and can express viral genes that induce cytotoxicity and immune responses. Moreover, transgene expression by recombinant HSV-1 vectors is usually transient. It may be possible in the future to harness two viral latency-active promoters to provide stable gene expression from HSV-1 vectors.

The HSV-1 amplicon vector system is based on the ability of HSV-1 to package defective genomes containing the *cis*-acting sequences *ori* (origin of viral DNA replication) and *pac* (packaging and cleavage signal). Besides these *cis*-acting elements, no other viral genes are retained in HSV-1 amplicon vectors (99). However, packaging of the amplicon vectors requires a replicating helper virus, which can result in high-level contamination with a replication-competent virus. This problem has been overcome by the development of a helper-free packaging system in which viral genes are provided in *trans* from cosmids or from a bacterial artificial chromosome lacking the *pac* signal.

Transduction with HSV vectors has been demonstrated in a large number of cell types, and these vectors have been applied to multiple gene therapy strategies, including neurological diseases, spinal nerve injury, glioblastoma, and even pain therapy (95). Sensory neurons can be infected by direct interdermal injection of the vector, and the DNA can persist in the nerve cell body. Maintaining high gene expression levels over long periods of time is a problem in certain cell types, such as the brain. The major limitations for recombinant HSV-1 vectors are their cytopathic effect and the induction of an immune response by viral gene expression. The development of amplicon vectors and a helper virus-free packaging system has overcome this problem (99). However, additional deletion of nonessential genes from the *bac* packaging system may also be necessary to prevent cytotoxicity and recombination within this vector system. The large packaging capacity of HSV-1 amplicons (up to a theoretical 152 kb) may be very useful for delivering complex genes and regulatory sequences or multiple copies of the transgene.

VECTOR TROPISM AND THE SPECIFICITY OF TRANSDUCTION

Although recombinant vectors have demonstrated wide host ranges, this may be disadvantageous for systemic gene therapy approaches. Transduction of cell types other than the target can have detrimental effects and diminishes the impact and effectual dose of the vector. Targeted delivery to the appropriate cell type would remove these risks and allow lower vector doses to be administered. In addition, there are cells that are refractory to transduction owing to poor expression of the endogenous cellular receptor. Various attempts have been made with different vector systems to alter the host range (100, 101). Among the strategies that have been explored is the approach of pseudotyping with elements from different viruses. Chemical attachment of ligands has been used to impart specific binding characteristics to purified vector virions. Although cell-specific targeting can be achieved by this approach in vitro, the stability of the complexes may not be sufficient to achieve targeting in vivo. Direct genetic modification is an attractive approach to introduce targeting ligand peptides into viral proteins, which mediates binding with alternative cellular receptors. Often the major challenge is to maintain high infectivity for modified vectors.

Retroviruses have been retargeted by fusion proteins of the envelope with single-chain variable antibody fragments to form a bispecific protein that binds to a specific cell surface protein (102). Ligands have also been introduced as extensions to the native surface (SU) glycoprotein (103). Although these vectors have demonstrated altered tropisms, they tend to exhibit low levels of infectivity.

Recombinant adenoviruses have been generated from a number of different serotypes, and these show variations in cell tropism (104). Pseudotyping with different fiber proteins is sufficient to alter tropism (105, 106). Viruses have also been engineered to express chimeric fiber proteins (107). Targeted Ad vectors are likely to hold more promise for systemic gene delivery and should possess reduced immunogenicity and toxicity, and hence increased safety (108). Bispecific proteins have been generated to retarget the vector to a number of cell types by fusing antibody fragments to the virus (normally to the fiber knob) together with targeting ligands (109). Targeting peptides have also been introduced directly into the fiber knob. Increased transduction of a number of cell lines devoid of high levels of the CAR was demonstrated with viruses containing fiber fused to RGD (110) or polylysine sequences (111). Nontarget transduction can be reduced by ablating the natural binding to the CAR and integrin receptors (112).

The simplicity of the structural elements of the AAV capsid makes it an attractive vector system for retargeting (113). Although AAV2 displays a broad host range, certain cell types are resistant to AAV2 infection, probably owing to the lack of appropriate receptors. Differences in cellular tropism have been observed for viruses using the capsid proteins from serotypes other than AAV2, which use alternative receptors for cell attachment and entry (114–116). AAV1 capsids show the highest transduction efficiency in muscle and liver, whereas AAV5 displays high tropism for retina (115, 117, 118). Furthermore, AAV5 is able to transduce airway epithelia cells (119), and AAV3 shows tropism for hematopoietic stem cells (120), which are resistant to transduction by AAV2. Even with a single organ there may be differential transduction of specific cell types (116). Mixing different capsid proteins suggests that it is possible to generate chimeric vectors that impart novel transduction phenotypes compared to either parental capsid alone (121). Sequence analysis of the cap proteins reveals regions with considerable diversity that are likely to be responsible for different tropisms (59). Altering the tropism of AAV vectors has also been explored by chemical cross-linking of bispecific antibodies to the viral capsid (122) and by the insertion of receptor-specific epitopes in the cap proteins (123–126). Library approaches may allow the most efficient targeted vector to be selected from a pool of viruses containing ligands, after transduction of the cell type of choice (127, 128).

VECTOR RECOGNITION, PROCESSING, AND INTEGRATION

The recognition and processing of vector genomes will be important for determining the efficiency of transgene expression and, therefore, ultimately the success of the gene therapy approach. Integrating vectors provide a means to achieve long-term gene expression, assuming that there is no silencing of transcription from the inserted DNA. Associated with integration into the host cell genome will be the risk of disrupting an essential gene or misregulating transcription. Insertional mutagenesis by vector DNA has long been recognized as a potential hazard of gene therapy, and recent clinical studies have highlighted this danger. Although many of the vector systems being used do not integrate, the delivered transgene can be expressed over long periods of time from vector DNA that persists extrachromosomally. Processing by cellular machinery can produce structures that are stably maintained, and elements can also be rationally incorporated into vectors to promote episomal maintenance.

For retrovirus infections, integration into the host genomes is a necessary step in the virus life cycle (129, 130). It occurs with high efficiency but without clear preference for specific target sequences or loci. The viral integrase protein mediates the joining reaction, with cellular DNA repair proteins also implicated at various stages (131). The precise mechanisms used to determine the selection of the integration remain unknown. Until recently it was thought that retroviral integration occurred randomly and that the risks of cancer induction from a single retroviral integration event was negligible. The availability of the human genome sequences has enabled genome-wide analysis of integration site usage for different retroviruses (132). These studies have revealed that different retroviruses may have quite varied preferences for integration sites in human chromosomes. HIV strongly favors active genes (133), whereas MLV favors integration near transcription start regions and only weakly favors active genes (132). In contrast, the avian sarcoma-leukosis virus shows only a very weak bias toward integration in active genes and no favoring of integration near transcription start sites. These observations suggest that integration site selection is affected by different interactions at the integration site and that tethering through protein-protein interactions may play a role. This raises the possibility that a particular safe site within the genome might be favored for integration through fusion proteins that tether the integrase to a specific target sequence (134).

AAV vector dsDNA genomes can persist in transduced cells for long periods of time by either integration or extrachromosomal maintenance. The frequency of rAAV integration is quite low (135, 136), and therefore, the associated risks may be much reduced when compared with retrovirus vectors. The wild-type AAV virus has a unique ability to integrate its DNA in a site-specific manner into a locus on human chromosome 19 (57). Although this has been shown in vitro, there is no evidence for site-specific integration from human samples. Targeted integration requires the viral Rep protein, and as this is deleted from most rAAV vectors, the targeting is lost. Recent genome-wide studies with rAAV vectors have shown a preference for integration into genes compared to noncoding regions in vivo (136). Although the precise mechanism of rAAV integration is unclear, it involves cellular pathways of recombination and may take place at naturally occurring chromosomal breakage sites (135).

Recombinant AAV vectors can also be detected in high-molecular-weight DNA molecules that are thought to represent concatamers formed by head-to-tail recombination of the ITRs. Inside the nucleus of infected cells, the rAAV genomes are somehow recognized and processed into circles and concatemers that can continue to express the transgenes for long time periods (137). Although it is unclear which cellular factors are involved in the process, studies in severe combined immunodeficiency (SCID) mice suggest that repair proteins may influence the episomal structure of rAAV genomes and circle formation (138, 139). There is also evidence that cellular DNA repair proteins can associate directly with the AAV ITR, and their relative binding may influence processing and transduction (140).

Although the genomes of Ad and HSV-1 are not normally integrated during virus infection, engineering of viruses can promote persistence of the vector genomes. For example, incorporation of elements from the Epstein-Barr virus (*oriP* and EBNA-1 protein) has been demonstrated to aid maintenance of the viral DNA and prolong transgene expression in both Ad and HSV-1 amplicon vectors (141, 142). Large DNA viruses can also be used as carriers for delivery of integrating viruses in hybrid vector systems. The targeting ability of the Rep protein of AAV can be harnessed for site-specific integration from hybrid vector systems that contain a transgene flanked with AAV ITRs. Hybrids of AAV elements within Ad (143, 144) and HSV-1 (145, 146) vectors have been developed with this goal in mind. In addition, integration into specific predetermined sites may also be achieved with other virus-derived recombinases, such as ΦC31 bacteriophage (147).

Gene Regulation

For gene therapy to become a successful modality of modern medicine, it will be necessary to regulate expression of the transgene to the appropriate level. Most vector systems utilize strong constitutive viral promoters to achieve high levels of transgene expression. However, systems have been developed that utilize tissue-specific transcriptional regulation or regulatable transcriptional elements that can be switched on and off via exogenous stimuli. Tissue-specific promoters are often much weaker than the heterologous viral promoters, but transgene expression can also be controlled at the level of translation by inclusion of *cis*-acting post-transcriptional regulatory elements (PREs). The PREs most commonly used are derived from viral transcripts, and the PRE from the woodchuck hepatitis virus has been shown to increase reporter gene expression at least fivefold in viral vectors (148, 149).

The regulatable systems used in viral vectors are either based on naturally occurring inducible promoters that exhibit tissue specificity or consist of chimeric systems engineered from various prokaryotic or eukaryotic elements (see Reference

150 for a review). Among the chimeric regulatable systems, the tetracycline (tet) regulatable system (151) is one of the best characterized and most widely used systems. It is based on binding of the tet repressor of *Escherichia coli* to the tet operator sequence and has been developed to give transactivation in the presence or absence of tet (152). Single-vector systems have been developed to deliver the transgene under control of the inducible promoter together with the gene encoding the regulator (153–155).

Another family of chimeric regulated systems is based on steroid hormones and their nuclear receptors. Systems have been developed on the basis of hormones and their receptors from insects and mammals. The progesterone system is based on a mutated human progesterone receptor, which is activated by the antiprogesterone mifepristone (RU486) but not the endogenous molecule (156). The use of the insect ecdysone-responsive systems receptor system has the potential advantage that the ecdysone hormones are neither toxic nor known to affect mammalian physiology. Systems based on both the *Drosophila* and *Bombyx* ecdysone receptors have been developed (157, 158) and shown to function in viral vectors.

Chemically induced dimerization can be used as a way to control the activity of a bipartite transcription factor (159). Inducible dimerization based on binding to rapamycin has been used to regulate transcription in retroviral vectors (160), with low basal expression levels and high dose-dependent induction of transgene expression. In vivo regulation of gene expression using this inducible system has also been demonstrated after intramuscular injection of AAV vectors (161, 162).

Another means of regulating gene expression is through the development of designer transcription factors. Modular DNA-binding protein domains can be assembled to recognize a given sequence of a DNA in a regulatory region of a targeted gene (163). Transcription factors can then be engineered by linking the DNA-binding protein to a variety of effector domains that mediate transcriptional activation or repression. Zinc-finger protein transcription factors (ZFP-TFs) have been designed to control the expression of any desired target gene and, thus, provide potential therapeutic tools for the study and treatment of disease. For instance a ZFP-TF can repress target gene expression with single-gene specificity within the human genome. HIV-1 transcription and genes, including Chk2, ErbB2, and VEGF, have been regulated by this approach (164).

Unfortunately, there are no gene regulatory systems that have yet received U.S. Food and Drug Administration (FDA) approval for clinical use. In addition to these approaches for regulating gene expression, the emerging small interfering RNA technologies are likely to become an integral component of successful gene therapy strategies.

CLINICAL TRIALS: SUCCESSES AND SETBACKS

Worldwide, over 600 clinical trials using gene therapy have been conducted or are underway, with the enrollment of thousands of patients (see Figure 3). A substantial portion of these trials (over 70%) are cancer related and are often carried out using

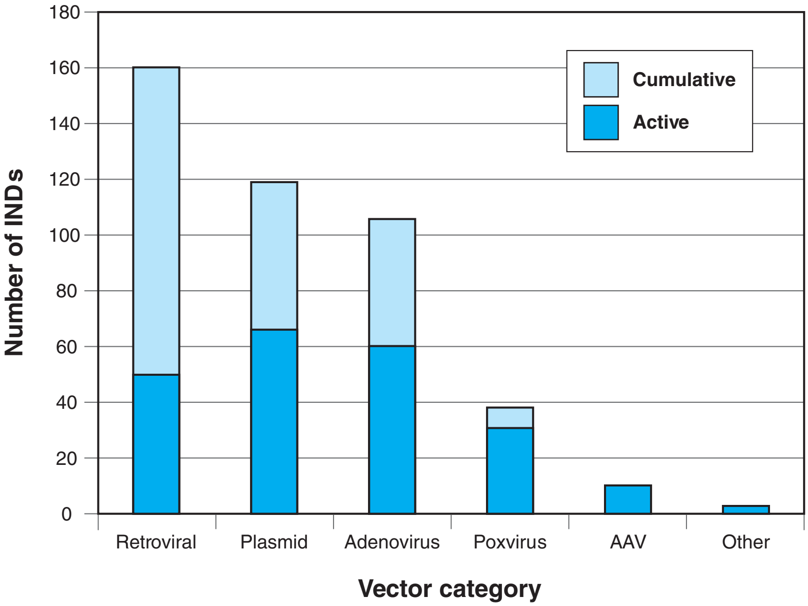

Figure 3 Clinical trials in gene therapy. A breakdown of the number of Investigational New Drug Applications relating to gene therapy as of March 2004, categorized according to the mode of delivery.

terminal patients. Most of the clinical trials are in Phase I or II, with less than 1% in Phase III, and therefore, there are presently no commercially approved gene therapy treatments.

Gene transfer into multipotent hematopoietic stem cells has received much attention because of its relevance for a broad variety of human diseases, ranging from hematological disorders to cancer (165). Retroviral vectors based on MLV were the first viral vectors to be used in a gene therapy trial and continue to be the most used. The first clinical trial attempted to treat SCID patients suffering from adenosine deaminase (ADA) deficiency, using retroviral vectors to transduce T lymphocytes (166). This study failed to show a long-term beneficial effect even though long-term reconstitution from transduced progenitor cells was observed at low levels. In addition to the transduced cells, these patients received ADA enzyme preparations, which might have prevented the selective outgrowth of the transduced progenitor cells. Although T-cell counts and function remained within normal limits, a loss of B and natural killer (NK) cells was observed in these patients, and ADA production was insufficient, probably owing to the limited number of transduced cells or low ADA expression levels.

The most successful trial that caught the attention of practioners of gene therapy and the general media was by Alan Fischer on children suffering from a fatal form of SCID, SCID-X1. This disease is an X-linked hereditary disorder characterized by an early block in the development of T and NK cells because of mutations in the γc cytokine receptor subunit. Hematopoietic stem cells from patients were stimulated and transduced ex vivo with an MLV-based retroviral vector, expressing the γc cytokine receptor subunit, and then were reinfused into the patients (167). During a 10-month follow up, γc-expressing T and NK cells could be detected, and cell counts and function were comparable to age-matched controls. The selective advantage of the γc-expressing lymphocyte progenitors contributed considerably to the success of this study. Unfortunately, three years after therapy was completed two of the children developed T-cell leukemia (168). In blood samples from these children, the leukemic cells contained a single intact copy of a retroviral vector that had integrated in or near the LMO2 gene (169). Although the precise mechanism by which LMO2 activation may cause T-cell leukemia remains conjectural, it is likely that the event is triggered by retroviral vector insertion. These results have rekindled the debate on the safety of gene therapy vectors.

The development of lentiviral vectors, which are able to transduce a hematopoietic progenitor in the absence of cytokines, might further improve stem cell gene therapy. Although these vectors have not yet been approved for use in clinical trials, some remarkable results have been obtained in animal models. Lentiviral vectors were successfully used to introduce a functional β-globin gene into hematopoietic stem cells and corrected β-thalassaemia and sickle-cell disease in mice models (170, 171). Furthermore, lentiviral vectors hold great promise in the treatment of neurological diseases, as demonstrated in a rhesus monkey model for Parkinson's disease (172) and a mouse model for metachromatic leukodystrophy, a lysosomal storage disease affecting the central nervous system (173).

In another clinical study, patients suffering from hemophilia B, a bleeding disorder caused by a deficiency of coagulation factor IX, were treated with AAV vectors expressing human factor IX (174). These patients participated in a Phase I trial and received intramuscular injections of AAV vectors. Although only very low levels of secreted factor IX could be detected in the plasma of one patient, the treated patients showed some clinical benefits and a reduced intake of factor IX infusions. Moreover, no vector-related toxicity and germline transmission was observed. These trials have now been abandoned in favor of injecting AAV factor 1X vectors directly into liver, which in turn have shown some unexplained toxicity.

One of the earliest hopes of gene therapy approaches was the possibility of using viral vectors to either introduce a lethal gene to cancer cells or to boost the immune system so as to recognize the tumor cell as foreign [some approaches and progress are reviewed in (175, 176)]. In addition to using viruses to deliver tumor suppressors, apoptotic inducers, suicide genes, and cytokines, another attractive approach to cancer gene therapy is to harness the lytic action of replicating viruses for tumor-specific killing (177). Viral oncolysis can be achieved with RNA viruses and also with DNA viruses such as Ad and HSV-1 (178–180). Restrictive growth in cancer cells can be combined with delivery of a therapeutic or toxic gene, and in addition, the immunogenic properties of Ad vectors can elicit an indirect antitumor effect. Promising results have emerged from Phase II clinical trials using intratumoral administration of oncolytic viruses in combination with standard chemotherapy.

Clinical trials involving gene therapy are closely monitored, and any adverse event is extensively scrutinized. In 1999, the University of Pennsylvania initiated a human Phase I clinical trial for the treatment of patients with deficiencies in ornithine transcarbamylase. This trial was designed to test the safety of an E1/E4-deleted recombinant adenovirus vector (181). An 18-year-old volunteer in the study, Jessie Gelsinger, received the highest dose and four days later became the first person to die as a result of vector delivery (182). This was heralded as a death of gene therapy, but as in many experimental therapies, it is hard to anticipate unexpected pitfalls. The community of gene therapy has learned some valuable lessons from this tragedy, particularly the value of reporting all adverse events and clear definition of clinical end points. As a result of this tragedy, there is also better coordination between regulatory agencies.

PERSPECTIVES: WHAT IS NEXT?

Rarely comes along a modality of medicine that has the prospect of such widespread and profound influence on human health. The young field of gene therapy promises major medical progress toward the cure of a broad spectrum of human diseases, ranging from immunological disorders to heart disease and cancer. It has, therefore, generated great hopes and great hypes, but it has yet to deliver its promised potential. The idea to use the genetic information obtained by sequencing of the human genome for the treatment of diseases is compelling. However, if scientists from many different disciplines participate and pull together as a team to tackle the obstacles, gene therapy will be added to our medicinal armada and the ever-expanding arsenal of new therapeutic modalities. Geneticists are required to identify target genes that contribute to specific diseases or to identify those that can influence the course of disease. The task for virologists is to develop efficient and safe vectors that are able to deliver the genes of interest to the target cells and that assure the proper expression of the transferred genetic material. Cell biologists will establish ways to facilitate the gene transfer and will identify stem cells that may be used to regenerate failing organs. Bioengineers are needed to show the biologists how three-dimensional tissues and even whole organs may be generated in a test tube. Clinicians will ultimately carry out clinical trials with vectors optimized for the disease and for the medical requirements of the patients.

Gene therapy has suffered highs and lows in both the public and scientific communities. It has undergone extreme scrutiny in the recent past. It is our responsibility to assure the public that the patients' welfare and health is the major goal. Strict adherence to the guidelines is incumbent on all scientists and investigators involved in a clinical trial. The gene therapy community will need to meet the challenge of new regulations and guidelines introduced by the National Institutes of Health (NIH) and FDA to ensure both the quality of clinical trials and protection of volunteers enrolled in the trials. Together researchers and clinicians will be able to continue to participate and lead the nation in harnessing natural biological processes to provide real therapeutic benefits in this unprecedented golden age of biomedical research.

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